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Paeonia anomala 的核型研究

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摘要: 报道了 *Paeonia anomala* L. 的核型, 澄清了新疆阿尔泰地区分布的该物种的染色体数目。分布于该地区的 *Paeonia anomala* L. 的核型组成: 2n = 2x = 10 = 6m + 2sm + 2st。该类群核型与该属其它类群一致——2A 型。在综合比较分析该属染色体参数以及核型不均一性参数, 包括最长 最短染色体比 (L_1/L_n) 和染色体不对称系数 (CKOA) 的基础上, 我们发现该属三个组在核型上没有明显分化, 仅在木本类群 (Sect. *Moutan* DC.) 和草本类群 (Sect. *Onaepia* Lindley 和 Sect. *Paeonia*) 之间存在微小差异。此外, 作为二倍体类群, 新疆阿尔泰地区分布的 *Paeonia anomala* L. 很可能是二倍体杂种, 这将为研究被子植物的父母本同倍化杂交式物种起源提供一个很好的研究材料。

关键词: *Paeonia*; *Paeonia anomala*; 核型; 染色体不对称系数

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Karyotypic Study on *Paeonia anomala* (Paeoniaceae)

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Abstract: This is the first report of the chromosome karyotype of *P. anomala* from the Altai populations. The populations sampled from the Altai region of Xinjiang, China, are diploid (2n = 10), with the 2A type karyotype and the karyotypic formula of 2n = 2x = 10 = 6m + 2sm + 2st. Based on karyotypic and asymmetric parameters, i.e., mean arm ratio, ratio of longest shortest chromosomes (L_1/L_n), coefficient of karyotypic overall asymmetry (CKOA), it was found that there was no apparent differentiation in karyotype features among all the three sections of the genus *Paeonia*, although some slight karyotypic differences were found between the woody section (Sect. *Moutan* DC.) and the herbaceous sections (Sect. *Onaepia* Lindley and Sect. *Paeonia*). The diploid status of the *P. anomala* populations, revealed in this study, implies that the species, assumed as interspecific hybridization origin, may add to the handful examples of homoploid hybrid speciation in angiosperms.

Key words: *Paeonia*; *Paeonia anomala*; Karyotype; Coefficient of Karyotypic Overall Asymmetry (CKOA)

The genus *Paeonia* L., with chromosomes large in size but low in number, has attracted considerable cytologists′ and cytogenetists′ attention since the 1930′s. The genus is divided into three sections, *Moutan* DC., *Onaepia* Lindley and *Paeonia*, with a total of ca. 30 species (Hong *et al*, 2001) distributed in the Northern Hemisphere. Among the species cytologically studied, all of those in sects. *Moutan* and *Onaepia* are

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diploid (2n = 10, Dark, 1936; Stebbins *et al*, 1938 and 1939; Stern, 1946; Delay, 1947; Gajewski, 1948; Okada *et al*, 1979; Li, 1982), while those in Sect. *Paeonia* are either diploid or tetraploid (2n = 20) (Dark, 1936; Stern, 1946; Sopova, 1971; Hong *et al*, 1988), except two species where both diploid and tetraploid were found intraspecifically (Sopova, 1971; Hong *et al*, 1988). However, due to lack of a universal parameter to measure the karyotypes, not all species reported have been sufficiently analyzed karyotypically so far. Among the well-analysed species the comparisons were made mostly between diploids and tetraploids, or between two sections (Tzanoudakis, 1983; Punina, 1987; Hong *et al*, 1988; Zhang and Hou, 1996).

Paeonia anomala L. is distributed in Xinjiang of northwestern China, northeastern Kazakhstan, and northern Mongolia and eastern of Russia (Hong *et al*, 2001). The accessions from northern Xinjiang were once misidentified as *P. sinjiangensis* Pan (Pan, 1979) but later were replaced (Hong, 2004). In the molecular systematic studies, Sang and Zhang (1999) speculated that the populations of *P. anomala* (under the name of *P. sinjiangensis*) distributed along Altai Mountains, Xinjiang, were interspecific hybrids. Because the Altai populations showed heterozygosity at nuclear genes, *Adh*1 and *Adh*2, and were well isolated with a sympatric diploid species, *P. intermedia* (Hong *et al*, 1994), they suggested that the Altai populations of *P. anomala* (*P. sinjiangensis*) were possibly allotetraploid, although the ploidy level Xinjiang's populations of *P. anomala* was then not known. This paper is to report the chromosomal number and the karyotype of the Altai populations of *P. anomala*.

Materials and Methods

Two populations of *Paeonia anomala* L. involved in this study were collected from Altai District, Xinjiang, China. One population (XJ021 and XJ027) was collected from Hanasi Nature Reserve (喀纳斯自然保护区), Buerjin County (布尔津县), and another (XJ034) from Baihaba Holiday Village (白哈巴渡假村), Habahe County (哈巴河县). All the vouchers are preserved in the Herbarium, Institute of Botany, the Chinese Academy of Sciences, Beijing (PE).

Flower buds used for chromosome study were fixed by Carnoy's solution Ⅰ (absolute ethanol: acetic acid = 3∶1) in middle of May, 2002. After fixation, the flower buds were transferred into 70% alcohol for long-term preservation. The chromosome slides were prepared using conventional squashing methods and stained by modified Carbol fuchsin. The micrographs were taken under Leitz Orthoplan microscope using 100x oil lens. At least five cells were calculated from each individual, and the results were shown in Table 1 and 2. The classification of karyotypes followed Stebbins (1971). Additional parameters measuring karyotype asymmetry, i.e., Coefficient of Karyotypic Overall Asymmetry (CKOA) and longest chromosome shortest chromosome ratio (L_l/L_n) were calculated according to Zhang (1998).

Table 1 The parameters of chromosomes in *Paeonia anomala*

No. Population	Chrosome number		Absolute. length (μm)	Relative length	Arm ratio	Chr. Type*
Population 1: XJ021	I	1	13.35±2.09	14.61 + 10.96 = 25.57	1.367±0.237	m
		2	11.00±2.05	12.36 + 8.71 = 21.07	1.370±0.267	m
		3	9.90±1.72	10.39 + 8.58 = 18.97	1.228±0.263	m
	II	4	9.68±1.29	12.66 + 5.87 = 18.53	2.158±0.224	sm
	III	5	8.28±0.85	12.17 + 3.69 = 15.86	3.300±0.307	st
Population 1: XJ027	I	1	12.22±1.21	13.63 + 11.05 = 24.68	1.244±0.169	m
		2	10.39±0.76	11.99 + 9.05 = 21.04	1.346±0.239	m
		3	9.25±0.58	10.40 + 8.36 = 18.76	1.241±0.137	m
	II	4	9.14±0.41	12.29 + 6.26 = 18.55	1.959±0.191	sm
	III	5	8.36±0.44	13.03 + 3.94 = 16.97	3.374±0.640	st
Population 2: XJ034	I	1	11.73±1.67	13.51 + 11.21 = 24.72	1.219±0.193	m
		2	10.31±1.08	11.74 + 10.04 = 21.78	1.178±0.129	m
		3	9.20±1.44	10.69 + 8.69 = 19.38	1.242±0.158	m
	II	4	8.41±1.05	11.32 + 6.40 = 17.72	1.821±0.330	sm
	III	5	7.79±1.32	11.85 + 4.55 = 16.40	3.088±0.303	st

* Chr. Type: Chromosome type, classified according Stebbins (1971)

Table 2 The karyotypic characters of the species reported in three sections of *Paeonia*

Taxon	L_1/L_n	Karyotype formula	Type	CKOA	RF
Sect. *Moutan*: Subsect. *Vaginitae*					
P. ostii T. Hong & J. X. Zhang	1.4	2n = 2x = 10 = 6m(3SAT) + 2sm(1SAT) + 2st(2SAT)	2A	0.248	Zhang and Hou, 1996
* *P. jishanensis* T. Hong & W. Z. Zhao	1.4	2n = 2x = 10 = 6m(2SAT) + 2sm(2SAT) + 2st(2SAT)	2A	0.247	Hong *et al*, 1988
* *P. decomposita* subsp. *decomposita*	1.4	2n = 2x = 10 = 6m + 2sm + 2st	2A	0.233	Hong *et al*, 1988
P. suffruticosa	1.4	2n = 2x = 10 = 6m(1SAT) + 2sm(1SAT) + 2st(2SAT)	2A	0.221	Hong *et al*, 1988
Sect. *Onaepia*					
P. californica	1.3	2n = 2x = 10 = 6m + 2sm + 2st(2SAT)	2A	0.250	Stebbins and Ellerton, 1939
P. brownii	1.8	2n = 2x = 10 = 6m + 2sm + 2st(2SAT)	2A	0.273	Stebbins and Ellerton, 1939
Sect. *Paeonia*: 1. subsect. *Paeonia*					
P. anomala (XJ021)	1.6	2n = 2x = 10 = 6m + 2sm + 2st	2A	0.245	In the text
P. anomala (XJ027)	1.5	2n = 2x = 10 = 6m + 2sm + 2st	2A	0.230	In the text
P. anomala (XJ034)	1.5	2n = 2x = 10 = 6m + 2sm + 2st	2A	0.198	In the text
P. lactiflora	1.5	2n = 2x = 10 = 6m(2SAT) + 2sm(1SAT) + 2st(1SAT)	2A	0.214	Hong *et al*, 1988
P. veitchii (PB85091-1)	1.6	2n = 2x = 10 = 6m(4SAT) + 2sm(1SAT) + 2st(2SAT)	2A	0.224	Hong *et al*, 1988
P. veitchii (PB85065)	1.5	2n = 2x = 10 = 6m(1SAT) + 2sm(1SAT) + 2st(1SAT)	2A	0.274	Hong *et al*, 1988
P. clusii ssp. *clusii* (P.1)	1.5	2n = 2x = 10 = 6m(1SAT) + 2sm(1SAT) + 2st(1SAT)	2A	0.232	Tzanoudakis, 1983
P. clusii ssp. *clusii* (P.4)	1.5	2n = 4x = 20 = 12m + 4sm + 4st(1SAT)	2A	0.230	Tzanoudakis, 1983
P. peregrina (P.27)	1.9	2n = 4x = 20 = 12m(1SAT) + 4sm(1SAT) + 4st(2SAT)	2A	0.232	Tzanoudakis, 1983
2. Subsect. *Foliolatae*					
P. mairei (PB85023-2)	1.6	2n = 4x = 20 = 12m + 4sm + 4st(4SAT)	2A	0.223	Hong *et al*, 1988
P. obovata (PB85078)	1.6	2n = 2x = 10 = 6m(2SAT) + 2sm(2SAT) + 2st(2SAT)	2A	0.263	Hong *et al*, 1988
P. obovata (PB85068)	1.9	2n = 4x = 20 = 12m + 4sm(1SAT) + 4st(3SAT)	2A	0.217	Hong *et al*, 1988
P. obovata (PB8524-4)	2.8	2n = 4x = 20 = 12m + 4sm + 4st(4SAT)	2B	0.175	Hong *et al*, 1988
P. mascula ssp. *mascula* (P.15)	1.5	2n = 4x = 20 = 12m + 4sm + 4st	2A	0.234	Tzanoudakis, 1983
P. mascula ssp. *russoi* (P.18)	1.5	2n = 2x = 10 = 6m(2SAT) + 2sm(1SAT) + 2st(2SAT)	2A	0.229	Tzanoudakis, 1983

L_1 = the longest chromosome; L_n = the shortest chromosome; CKOA: Coefficient of Karyotypic Overall Asymmetry; RF: Reference

*: *P. decomposita* subsp. *decomposita*, in the original reference as *P. szechuanica*; *P. jishanensis*, in the original reference as *P. suffruticosa* var. *papaveracea*.

Results

The two populations studied are diploid, 2n = 10 (Fig. 1). The materials used for karyotypic analyses were XJ021 (Population 1), XJ027 (Population 1) and XJ034 (Population 2), representing two populations. The karyotype formulas are shown in Table 1 and 2. The difference between these two populations is found on chromosome 3 and chromosome 4 (Table 1).

The CKOA values were different in the two populations. In the two individuals of Population 1, the CKOA was 0.245 and 0.230, respectively, while that of Population 2 was 0.198. Generally, the karyotype of *P. anamala* was similar to the species previously reported in the genus, all belonging to 2A type. The chromosomes can be classified into three types: Type I: including chromosome 1 through 3, was metacentric (m). Type II: including only chromosome 4, was submetacentric (sm), and Type III: including only chromosome 5, was subtelocentric (st).

Discussion

This was the first report of the karyotype of the Altai populations of *P. anomala*. Because of the previous taxonomic confusion of this species, especially with *P. intermedia*, the study added valuable information to the completion of cytological data of the genus *Paeonia*.

We synthesized the karyotypic parameters of all reported species (Table 2), and found that the karyotypes were highly consistent in all the species of the genus. In addition, the arm ratio of Chromosome 1 of Sect. *Moutan* (1.43-1.60) is similar in all the species but does not overlap with that of Sect. *Paeonia* (1.12-1.37), agreeing with Hong *et al* (1988). Meanwhile, the arm ratio of Chromosome 1 in Sect. *Onaepia* (1.13-1.20) does not overlap with that of Sect. *Moutan* either. This result further confirms that the woody section (Sect. *Moutan*) has apparently differentiated from the herbaceous sections (Sects. *Paeonia* and *Onaepia*) with respect to the arm ratio of these

chromosomes. The differentiation in karyotypes of the genus also exists in the ratio of the longest chromosome/shortest one. In Sect. *Moutan*, the L_1/L_n value is 1.4, in Sect. *Paeonia* $L_1/L_n \geq 1.5$, while in Sect. *Onaepia*, L_1/L_n of *P. californica* is 1.3 and *P. brownii* is 1.8.

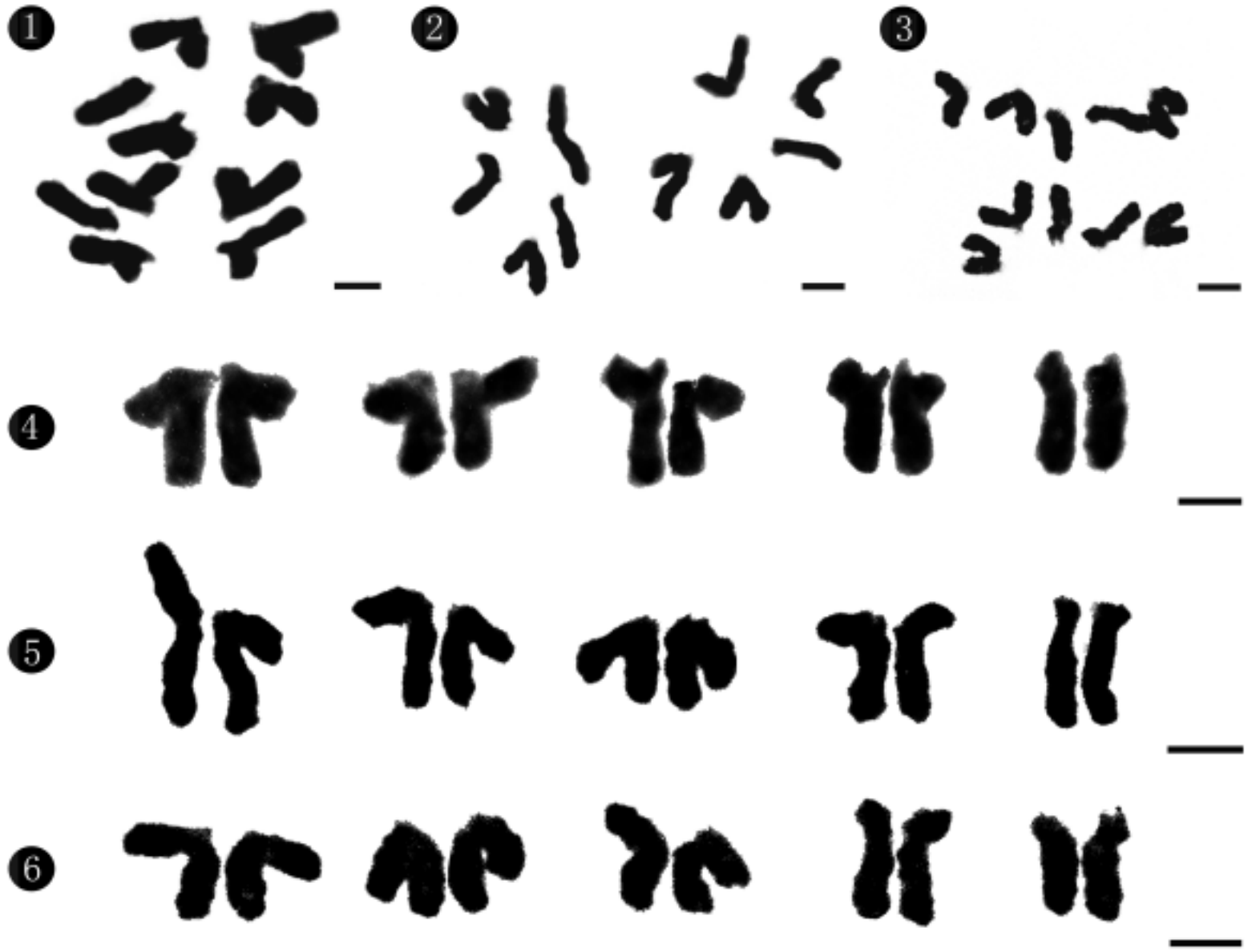

图 1 *P. anomala* **减数分裂后期 II 图。1, 4: XJ021; 2, 5: XJ027; 3, 6: XJ034. 4, 5, 6 分别是 1, 2, 3 对应的染色体组型图。标尺 = 5 μm**

Fig. 1 Meiotic anaphase II plates of *P. anomala*. 1, 4: XJ021; 2, 5: XJ027; 3, 6: XJ034. 4, 5, 6 is Karyogram of 1, 2, 3, respectively. Bar = 5 μm

In all the three sections of the genus, karyotypic asymmetry varies slightly, showing similar CKOA values (Table 2). However, from the meiotic studies (Sax, 1937; Stebbins and Ellerton, 1939; Walters, 1942; Zhang *et al*, 1997), a great deal of abnormal meiotic configurations was found in all the individuals observed, indicating that there existed many chromosomal structural variations in the genus. These phenomena are contradictory to the karyotypic consistence recognized in *Paeonia*. A better understanding of the contradiction requires further investigation. The study raises a very interesting question regarding to the evolutionary history of the Altai populations of *P. anomala*. The diploid status of the populations does not support the previous assumption of the allotetraploid origin of the species. The rather conclusive molecular phylogenetic evidence gathered so far, however, does point to a hybrid origin of the species (Sang *et al*, 1997; Sang and Zhang, 1999). The alternative hypothesis, therefore, is that *P. anomala* is a diploid hybrid that has happened to maintain heterozygosity at the *Adh* loci. Given that only handful examples of well-documented diploid hybrid species were reported so far in flowering plants (Rieseberg, 1997), this may represent an interesting case of homoploid hybrid speciation in *Paeonia*. The hypothesis needs further to test with phylogenetic data from more populations by nuclear genes of *P. anomala*.

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